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as Mtr4p play key roles in RNA metabolism. Mtr4p is thought to assist RNA degradation by the exosome presumably by unwinding RNA structures. In addition, the TRAMP complex appends short 3′ poly(A) tails to RNA meant for degradation by the exosome. Mtr4p within the TRAMP complex plays a crucial role in regulating the polyadenylation activity, limiting the poly(A) tail length. The unwinding activity of Mtr4p is required and indeed within the TRAMP complex, Mtr4p unwinds duplex RNA 10x faster than when alone. However, the dynamic details of the RNA unwinding activity and interaction of Mtr4p and TRAMP and indeed the Ski2-like family of RNA helicases are unknown. We have performed high-resolution RNA hairpin unwinding experiments for both Mtr4p and TRAMP. We find that Mtr4p unwinds RNA duplexes in a single or multiple bursts with a mean step size of 9 base pairs. The unwinding is irreversible suggesting that Mtr4p remains stably bound after unwinding the duplex, preventing re-annealing. TRAMP unwinding experiments reveal a faster molecular recognition by the Mtr4p helicase when in complex as well as enhanced RNA duplex unwinding dynamics. Combined high-resolution trapping and fluorescence experiments underway may reveal how the competition within TRAMP between the 3′-to-5′ Mtr4p helicase and the 5′-to-3′ Trf4p polymerase is resolved dynamically.

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Electrostatic Interaction Effects on the Binding of Spliceosomal U1A Protein-SL2 RNA Hairpin
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The U1A protein is a component of the U1 small nuclear ribonucleoprotein particle, which forms part of the spliceosome. Since U1A protein is highly charged, electrostatic interactions have been suggested to be of importance in the association of the protein with RNA and the stability of the complex. To understand this effect, positively charged residues of the protein have been mutated in three positions (K20, K22 and K50) that do not have direct interactions with the SL2 RNA.We performed extensive molecule dynamics simulations on the mutant complexes and characterized the changes in the networks of interactions.
To reveal more global variations within the mutant complexes, we also determined the changes on the subsections of the proteins-RNA (called communities) that move in concert together. These communities have been calculated based on generalized correlations to account for the collective atomic fluctuations within the protein-RNA complexes. These results are in agreement with the experimental complex dissociation binding studies.
We then determined a measure based on the collective atomic fluctuations of different mutant complexes that have a linear correlation with the experimentally measured dissociation constants. This correlation can be used to further predict the effect of a mutation on the dissociation constant of the U1A-SL2 RNA complexes.

73-Plat
Chromosome Reorganization by the Highly Cooperative Dps Protein
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All living creatures organize their genome into a dynamic three-dimensional nucleoprotein complex. This organization has a vital importance for cellular processes such as gene expression, DNA replication, and DNA repair. In order to understand the nature of chromosome architecture we need to gain insight into the biophysical parameters that drive compaction. However, critical details of this process remain unknown. In this study we investigate a DNA-binding enzyme from E. coli that rearranges the bacterial genome and protects the chromosome against cellular stresses ranging from starvation to antibiotic exposure. The enzyme responsible for this transformation is called Dps (DNA-binding protein from starved cells). The interaction between Dps and DNA results in the rapid formation of a tightly packed three-dimensional crystal lattice termed a biocrystal. Although static structures of biocrystals have been documented, little is known about how the structures form. We have developed a fluorescent assay for tracking the physical interaction between individual DNA molecules and Dps under TIRF microscopy. We found that Dps induces collapse extremely rapidly after a slow nucleation event. In addition, we have used magnetic tweezers to obtain biophysical parameters of the conformational transition of stretched DNA initiated by Dps protein. We found that cooperative Dps binding to DNA cannot be fitted with Hill equation but displays the qualitative features of an Ising system. Consequently we have developed a mean field model that captures important features of the collapse. These in vitro experiments provide the crucial details about the highly cooperative mechanisms of DNA-Dps biocrystal formation.

74-Plat
Asymmetric Nucleosome Disassembly with Disrupted Histones Revealed by Time Resolved Small Angle X-Ray Scattering with Contrast Variation
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DNA packages into compact chromatin structure in eukaryotic cell despite its high negative charge. Nucleosome core particles (NCP) are the fundamental repeating units of chromatin and modulate DNA accessibility for gene expression and regulation. NCPs contain a symmetric histone octamer wrapped by 147-bp DNA. The dynamics of DNA packaging and unpackaging from NCPs affects all DNA-based chemistries, but is not well understood due to a lack of structures of the partially unwrapped, kinetic intermediates. Here we applied a novel strategy combining contrast variation with time-resolved small angle x-ray scattering to determine the structures of protein and DNA constituents of NCPs during salt-induced disassembly. We monitored DNA conformation and protein dissociation of NCPs with two positioning sequences: Widom 601 and 5S DNA. For the Widom 601 construct, we measure a transient structure on the millisecond time scale where the DNA is asymmetrically released from a disrupted histone core, and the proteins remain bound to unwrapped DNA in a semi-open conformation. We hypothesis this conformation may be biologically important substrate for gene regulation. The 5S construct also displays asymmetric DNA release, but exhibits a different pattern of protein dissociation. Our results establish a powerful platform for studying the global dynamics of nucleoprotein complexes.

75-Plat
Understanding without Reading: Analogue Encoding of Physicochemical Properties of Proteins in their Cognate Messenger RNA
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Being related by the genetic code, messenger RNAs (mRNAs) and cognate proteins are polymers with mutually interdependent compositions, which further implies the possibility of a direct connection between their general physicochemical properties. How efficiently do different characteristics of mRNA coding regions reflect the features of cognate proteins and is it possible for the cell to obtain information about proteins from their mRNAs without first reading them on the ribosome? We address these issues in a theoretical proteome-wide analysis and show that average protein hydrophobicity, calculated from either sequences or 3D structures, can be encoded in an analogue fashion by many different mRNA sequence properties with the only constraint being that pyrimidine and purine bases be clearly distinguishable on average [1]. Moreover, average characteristics of mRNA sequences allow for a reasonable discrimination between human proteins with different cellular localization and, in particular, cytosolic and membrane proteins, even in the absence of topogenic signal-based mechanisms. We discuss our findings in the context of protein and mRNA localization and propose that this cellular process may be partly determined by basic physicochemical rationales and interdependencies between the two biomolecules.
[1] Polyansky AA, Hlevnjak M, Zagrovic B. (2013) Nat. Commun. 4, 2784.

Platform: Protein Structure and Conformation I

76-Plat
Role of Interfaces in Peptide Folding and Aggregation
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Protein misfolding is an intrinsic property of polypeptides, and misfolded conformations have a propensity to aggregate. In the past decade, the development of various coarse grained models for proteins have provided key insights into the driving forces in folding and aggregation. We recently developed a low resolution polarizable coarse grained model by adding oppositely charged dummy particles inside protein backbone beads. With this model, we were able to achieve significant alpha/beta secondary structure content de novo, without any added bias1. We now extend the model to study peptide aggregation at hydrophobic-hydrophilic interfaces and draw comparisons to aggregation in explicit water solvent. Elastin-like octapeptides (GV)4 are used as a model